REMARKS

A Request For A One Month Extension Of Time is submitted herewith, along with the required extension fee of $55.00.

A Declaration by inventor Brian L. Craine is submitted herewith to provide objective evidence in the record in support of various statements made herein.

In response to the Office Action, claims 1-6, 8, 10, 12 and 23 have been amended to more accurately define the invention and to be more grammatically correct. The specification also has been amended to correct various grammatical errors.

The examiner rejected claims 1-12 under 35 U.S.C. §112 , first paragraph, on the ground that the specification does not enable any person skilled in the art to practice the invention commensurate in scope with the claims, and also rejected claims 23 and 24 under 35 U.S.C. §112 on the grounds that the disclosure is not enabling. The examiner also rejected claims 1-24 under 35 U.S.C. 103(a) on various combinations of the Fielder et al., Benezra et al., Hacker, Brady et al., Machida et al., Schmitz, HYDROFLUOR-Combo, and "Manual for Biochem 651" references.

Reconsideration of each ground of rejection is respectfully requested in view of the amendments and arguments herein and the evidence submitted in the Craine Declaration.

The rejections under 35 U.S.C. 112:

Regarding claims 1, 5 and 9, the examiner contends that the specification does not enable any person skilled in the art to practice the invention commensurate in scope with the claims because no one of ordinary skill in the art can determine whether blood came from an upper or lower GI site on the basis of absorption spectra "if no preliminary data on which absorption peaks can be observed for both cases exist".

Regarding claims 2-12, the examiner contends that it is well known that the absorption lines of various hemoglobins and derivatives depend on the pH of their solutions and that the absorption peaks of hemoglobin will be substantially different than those disclosed for other pH values.

No Undue Experimentation Would Be Required for One Skilled in the Art to Practice the Claimed Invention:

A patent application meets the requirements of 35 U.S.C. 112, first paragraph, if it contains a written description of the invention which is sufficiently clear and complete to enable one of ordinary skill in the art to which the invention pertains to make and use the invention as claimed without undue experimentation.

As explained in paragraph 5 of the Craine Declaration submitted herewith, a person skilled in this art wanting to measure a test sample using spectroscopy ordinarily first obtains a

standardized spectrophotometric reading by using reference samples which are known forms of the unknown test sample.

As explained in paragraph 6 of the Craine Declaration, a person skilled in this art wanting to perform the method of the claimed invention in accordance with the description provided in the specification would ordinarily have a prepared reference samples having the same pH as the stool test sample and used the spectrophotometer to determine the peak values of the main Soret band and also to determine any visible additional absorption peaks of both pure hemoglobin (ferrous heme) and acid-treated hemoglobin (ferric heme) before using the same spectrophotometer to determine the values of corresponding peaks in the spectrum of the stool test sample.

As explained in paragraph 7 of the Craine Declaration, a person skilled in this art wanting to perform the method of the claimed invention in accordance with the specification could have easily prepared the reference samples in essentially the same manner described on page 15, lines 9-18 of the specification for preparing the stool test sample, except that the first reference sample would be prepared using pure hemoglobin instead of fecal matter and adjusting the pH to the same value as that of the stool test sample in order to determine the absorption peak of the main Soret band for pure hemoglobin and any additional visible peaks. The person skilled in the art could also have easily prepared a second reference sample using hemoglobin treated with acid similar to that present in the human stomach and adjusting the pH of the second sample to the same value as the pH of the stool test sample in order to determine the absorption peak of the main Soret band and any additional visible peaks for the acid-treated hemoglobin (ferric heme).

As explained in paragraph 8 of the Craine Declaration, that is how the present inventor obtained the reference spectra shown in Fig. 2 and mentioned in the specification on page 14, lines 10-18. That is how anyone of ordinary skill in the art having read the present specification and wanting to perform the method of invention could also have easily done it. It would have been rather pointless to run numerous experiments at different pH levels to provide data on absorption points as a function of pH because those skilled in the art wanting to practice the invention by using spectroscopy to analyze stool samples would have ordinarily routinely prepared and then spectroscopically analyzed corresponding reference samples for ferrous heme and ferric heme, respectively, as a matter of ordinary good spectroscopic analysis practice.

As explained in paragraph 9 of the Craine Declaration, the changes in the locations of the absorption peaks of ferrous heme and ferric heme are small over the meaningful pH ranges listed in subsequently described Table 1, but nevertheless, as a matter of standard good spectroscopy practice, the change ordinarily would be compensated for by measuring "standard" ferrous heme preparations and ferric heme preparations under the same exact conditions as the heme sample to be tested. For example, in a commercial version of the invention, a small tube of a standard ferrous heme preparation and a small tube of a standard ferric heme preparation would be provided for a user's convenience in preparing standard ferrous heme and ferric heme preparations having a pH corresponding to that of a particular buffer desired by the user for use in preparing a test stool sample, and then measuring, i.e., "standardizing", the ferric heme and ferrous heme reference absorption peak positions of the spectrophotometer for that buffer.

Therefore, it is respectfully submitted that any person skilled in this art having read the

application and wanting to practice the method of invention would have wanted to have the spectrophotometer calibrated and would have wanted to know the spectrophotometer readings of the absorption peaks of the particular substances in reference samples with which the test sample is to be compared, so the absorption peaks of the stool test sample can be meaningfully compared with the corresponding peaks of the reference samples. As a matter of nearly universal good spectroscopy practice, any person skilled in the art will prepare the reference samples exactly the same way (e.g., with the same pH) as the stool test samples except that the fecal material will be replaced by the reference substances, which in this case are pure (ferrous) hemoglobin and acid-treated (ferric) hemoglobin.

Furthermore, it is well known that the spectra of the two molecules (i.e., a ferric heme molecule and a ferrous heme molecule) are different. Therefore, any spectroscopic analysis of a test stool sample which concentrates those two molecules should be very predictable regardless of the wavelength at which the spectrophotometer operates. See paragraph 10 of the Craine Declaration.

Variation with pH from the Disclosed Wavelengths of the Absorption Spectra of the Stool Samples is Negligible over a Large pH Range:

As explained in paragraph 11 of the Craine Declaration and Table 1 thereof, Dr. Craine prepared reference samples of ferrous hemoglobin and ferric hemoglobin having pH values of 4.5, 6.8, 7.4 and 8.0 in essentially the same manner described on page 15, lines 9-18 of the specification, and used a spectrophotometer to determine the locations of the main Soret

absorption peaks. The results in Table 1, which is repeated below, show that there is no appreciable variation in the location of the Soret absorption peak of either ferric or ferrous hemoglobin in the useful pH range from 6.8 to 8.0.

Specifically, Table 1 shows measured wavelengths of the Soret peak locations at different pH values for ferrous heme and ferric heme. In each case there is very little change in the locations of the absorption peaks from a pH of about 6.8 to about 8.0. (Also, in each case, the peaks become difficult to differentiate at the lower pH of 4.5 .)

Table 1

Peak Wavelength of Soret Peak (nm)

Sample pH	Ferrous Hemoglobin	Ferric Hemoglobin
4.5	408.5	407.0
6.8	414.6	406.5
7.4	414.6	406.3
8.0	414.6	406.3

Furthermore, inventor Dr. Craine at the same time noted that the locations of the secondary "540 nm" and "576 nm" absorption peaks were not significantly changed for pH values of 6.8, 7.4 and 8.0. See paragraph 12 of the Craine Declaration.

The results in Table 1 show that the variation in the location of the Soret absorption peak

of ferric hemoglobin in the useful pH range from 6.8 to 8.0 is only 0.2 nanometers. This variation is not appreciable with respect to the present invention, because 0.2 nanometers is negligible compared to the much larger difference of approximately 8 nanometers in the location of the Soret absorption peaks of ferrous hemoglobin and ferric hemoglobin in the pH range from 6.8 to 8.0.

Therefore, as stated in paragraph 13 of the Craine Declaration, the locations of the Soret absorption peaks of 408 nanometers for ferrous hemoglobin and 415 nanometers for ferric hemoglobin set forth on page 14 of the specification for a pH of 7.4 are equally applicable for samples having a pH anywhere in the range from 6.8 to 8.0 for the purposes of the present invention.

Thus, even though one of ordinary skill in art would ordinarily have prepared reference samples in essentially the same manner as the stool test sample (as explained above) in order to obtain reference values of the absorption peaks of pure hemoglobin and acid-treated hemoglobin of the same pH as the stool test sample to be tested, Dr. Craine's actual measurements nevertheless show that the absorption lines of hemoglobin for values of pH between 6.8 and 8.0 do not vary enough to appreciably affect the results of performing the method of invention, so the results of performing the method for a pH of 7.4 are essentially the same over the much larger range of pH values from 6.8 to 8.0 even if control samples are not prepared to determine the absorption peaks of pure hemoglobin and acid-treated hemoglobin.

Specifically, the 0.2 nanometer variation of the absorption peak of the main Soret band of

pure hemoglobin having a pH of 6.8 and that having a pH of 8.0 is substantially less than the 8.0 nanometer shift of the corresponding absorption peak due to converting it to ferric heme by passing it through the acid environment of the human stomach. Therefore, performing the invention as claimed provides the same results regardless of whether absorption spectra of the test stool sample are compared with the disclosed reference values corresponding to a pH of 7.4 or with absorption spectra of ferric heme and ferrous heme reference samples prepared in essentially the same manner as the test stool sample.

Therefore, it is respectfully submitted that no undue experimentation would have been required by one skilled in the art to use conventional spectroscopy as a step in the method of the present invention based on the description provided in the specification as filed.

Regarding claims 23 and 24, the examiner contends that the disclosure is not enabling because specific IR wavelengths for detecting ferrihemes and ferroheme for various values of pH using the method of invention are critical to practice of the invention.

However, as explained above, one of ordinary skill the in the art could, and ordinarily would, easily and without any undue experimentation whatever, have been able to provide reference samples including ferric heme and ferrous heme in essentially the same manner as the test stool sample, determine the corresponding reference absorption peaks for the ferric heme and ferrous heme reference samples using an IR spectrophotometer, obtain the spectra of the test stool sample, and thereby readily determine by comparison of the test stool spectra and the reference samples spectra whether the stool test sample contains relevant amounts of ferrous

heme and ferric heme that are indicative of whether the heme in the test stool sample originated in the upper or lower GI tract. Furthermore, the nitrocellulose sample filter described in the application selectively binds and concentrates the heme material of the test sample so that it can be measured with any spectrometer, irrespective of the wavelength range of the spectrometer. The wavelength range of the spectrometer would not make a significant difference, and there is no practical reason why the spectrometer cannot be an IR spectrometer. See paragraphs 10, 14 and 15 of the Craine Declaration.

Therefore, it is respectfully submitted that if the reference samples and test sample were prepared in essentially the same manner, then performing the described method using an IR spectrophotometer would have been expected by those skilled in the art to provide the same results and conclusions regarding the location of the bleed site as if a standard spectrophotometer had been used.

In view of the foregoing considerations, it is respectfully submitted that one of ordinary skill in this art who had read the above application could have easily practiced the present invention as defined in all of the claims without undue experimentation. Therefore, it is respectfully requested that all of the rejections under 35 U.S.C. §112, first paragraph be withdrawn.

The rejections under 35 U.S.C. 103(a):

The present invention:

The present invention uses spectroscopy on a test sample prepared from a stool sample in a novel manner to determine (1) if there is sufficient ferric heme, i.e., ferriheme, in the sample to show that the hemoglobin products of the test stool sample were exposed to the acid environment of the human stomach, and (2) if there is sufficient ferrous heme in the test sample to show the presence of a bleed in the lower GI tract.

In the described embodiments of the invention, the stool sample is mixed with TE buffer because the TE buffer has a lower ionic strength than typical "biological conditions", and therefore causes red blood cells to swell and burst. This releases the hemoglobin and its degradation products into solution. (See page 18, lines 4-6 of the specification.)

The mixture of stool sample and TE buffer is centrifuged to remove stool particles (in contrast to the subsequently described Fielder reference, which discloses centrifuging and filtering to remove precipitated hematin rather than stool particles).

Then the supernatant is filtered to absorb and concentrate the heme components in a nitrocellulose sample filter and also to exclude contaminants such as urobilinogen which prevent meaningful spectroscopic analysis. The sample filter used in the present invention selectively binds and concentrates the heme material to be spectroscopically analyzed so that it can be measured with the spectrophotometer (irrespective of what the wavelength range of the spectrophotometer is). Specifically, the hydrophobic properties of the nitrocellulose filter

directly bind the porphyrin containing hemoglobin or hemoglobin breakdown products. Urobilinigen, the major contaminant in the stool sample, does not bind to nitrocellulose filters and is washed away to accomplish substantial purification of the sample to enhance the effectiveness of analysis by spectrophotometry. A suitable concentration of glycerol, which was determined after considerable experimentation, is used to wet the sample filter to make it more transparent to spectroscopic analysis.

Some of the hemoglobin breakdown products, including iron protoporphyrin, that are inherently included in the spectroscopic measurement in accordance with the claimed invention have lost their ability to have peroxidase activity. When samples of such breakdown products are acidified, they convert all of the ferrous heme to ferric heme. See paragraph 16 of the Craine Declaration.

The above application describes using conventional spectroscopy to evaluate the chemical condition of iron protoporphyrin rings of molecules that can be in a range of compounds ranging from native hemoglobin to heme which has been highly degraded by protease activity. The hemoglobin molecule has a substantial amount of protein folded around it which can be degraded a great deal without significantly affecting the iron protoporphyrin ring. The iron protoporphyrin ring is the part of hemoglobin module that holds an iron molecule. The main absorption peaks are caused by the iron protoporphyrin ring part of the hemoglobin molecule and the degraded derivatives of hemoglobin molecules. Consequently, as long as the porphyrin ring is still present, the main absorption of hemoglobin degradation products are essentially unchanged from those of the native hemoglobin molecule. See paragraph 17 of the

Craine Declaration.

The patent application describes determining the spectra of the iron protoporphyrin ring to measure whether the heme in the test stool sample is ferrous heme or ferric heme. The spectra indicate relative amounts of ferric vs. ferrous porphyrin rings which are part of the hemoglobin molecule or of a number of hemoglobin degradation products. The spectra is a summation of the spectra for ferric heme and ferrous heme, and the relative amounts can be determined by mathematical methods as set forth on pages 20 and 21 of the specification to determine the fraction of the individual spectra needed to cause the observed spectrum. Spectra are typically plotted as absorption versus wavelength, as in Fig. 2 of the specification, and those skilled in the art typically are able to visually determine the presence of ferric or ferrous forms by pattern recognition. For example the presence of the peaks at 540 nm and 576 nm are easily recognized features of ferrous forms of the molecule. The presence of a broad Soret band or a Soret band with a dip in the middle is recognized as a mixture of ferrous and ferric forms. The heights of the 540 nm peak or the 576 nm peak to the relative height of the Soret band may also serve as a visual cue for a mixture of ferrous and ferric forms. The position of the Soret band can also be visually determined to indicate a predominance of ferric or ferrous forms. See paragraph 18 of the Craine Declaration.

Note that hematin is included in, but is not specific to, the ferric heme detected by the present invention. (In contrast to the subsequently described Fielder reference, the present invention does not use detection of hematin as the differentiating factor between upper and lower GI bleeds. Note that the presence of such additional hemoglobin breakdown products cannot be

determined by measuring hemoglobin using the peroxidase activity test disclosed in the subsequently described Fielder reference.) See paragraph 19 of the Craine Declaration.

As indicated in paragraph 20 of the Craine Declaration, in the spectroscopic analysis described in the above patent application, the amount of hematin alone is not measured, and instead the presence of hematin and a number of hemoglobin breakdown products in addition to hematin are simultaneously measured. The spectroscopic analysis of the present invention looks at the porphyrin rings of hemoglobin molecules and additional hemoglobin breakdown products which may include hematin. (This is in contrast to the subsequently described Fielder reference, in which the presence of such additional hemoglobin breakdown products cannot be determined by measuring only hematin using the peroxidase activity test.)

As explained in paragraph 21 of the Craine Declaration, the method of the present invention does not specifically measure the presence of hematin, and instead detects the presence and relative amounts of all ferric hemes, including ferric hemes other than hematin. The measured degradation of hemoglobin in the present invention is dependent primarily upon acidic exposure of the hemoglobin in the stomach and is not significantly affected by bacterial action in the lower GI tract. The spectroscopic measurement in the present invention is dependent primarily upon presence or absence of acidic exposure of hemoglobin in the stomach and is not significantly affected by bacterial action in the lower GI tract. Consequently, the method of the present invention is believed to not exhibit an erroneous indication of an upper GI bleed due to hemoglobin products formed by bacterial action that could give an erroneous indication of an upper GI bleed in the method of Fielder and therefore is believed to be more reliable than the

prior art, especially the subsequently described Fielder reference.

Regarding claims 1 and 5, the examiner rejected them as obvious over the Fielder reference in view of the Hacker reference. **Regarding dependent claims 2-4 and 6-8,** the examiner rejected them as obvious over Fielder in view of Hacker and Brady.

The Fielder et al. Reference:

The Fielder reference discloses a method for indicating the presence of blood originating in the lower GI tract. The pH of a liquid stool sample is lowered to a value of 3.5 to 5.0 using an acidic buffer in order to cause precipitation of hematin. The resulting solution is filtered or centrifuged to remove any precipitated hematin. The filtrate or supernatant is tested for peroxidase activity to determine the presence or absence of hemoglobin. A positive result is interpreted to indicate bleeding in the lower GI tract. The Fielder reference fails to disclose any test for providing a positive indication of blood originating in the upper GI tract. See paragraph 22 of the Craine Declaration.

The pH of the solution used to prepare the test stool sample in the Fielder reference is very low, i.e., 3.5-5.0, and this would cause conversion of the ferrous heme to ferric heme and therefore would tend to greatly reduce or eliminate the difference between the wavelengths of the absorption peaks for ferric heme and ferrous heme. The acid environment to which the Fielder stool sample is subjected during preparation greatly compromises the ability of the Fielder method to provide a reliable conclusion that the subject stool sample has not been exposed to the

acid environment of the stomach. See paragraph 23 of the Craine Declaration.

The Fielder reference discloses precipitating the hematin and then measuring the resulting supernatant for peroxidase activity to determine if hemoglobin is present. See the "Summary of the Invention" in column 2, which makes it clear that Fielder et al. precipitate the hematin and then measure peroxidase activity of the remaining fluid to determine if it does contain any hemoglobin. If the remaining fluid still contains any hemoglobin, the Fielder reference concludes that the test sample must have originated in the lower GI tract. The Fielder reference seems to imply, but does not expressly state, that if there is hematin in the sample that has been prepared, then it must be the result of an upper tract bleed.

But a problem with the Fielder approach is that not all hemoglobin is converted by stomach acid to hematin in the upper GI tract. As indicated on page 13, lines 5 and 6 of the applicant's specification, some hemoglobin caused by bleeding in the upper GI tract passes unchanged into the lower GI tract, and could be detected by the peroxidase test of the Fielder reference and could cause a false positive identification of a lower GI bleed. See paragraph 24 of the Craine Declaration.

The Fielder technique of measuring the peroxidase activity in the supernatant is capable of only inaccurately indicating whether bleeding originated in the lower GI tract, but is not capable of determining whether bleeding occurred in the upper GI tract. In Fielder, only the presence of a significant amount of precipitated hematin would indicate the presence of an upper GI bleed, but there is no indication in Fielder what constitutes a sufficient amount of precipitated

hematin to indicate an upper GI bleed. See paragraph 25 of the Craine Declaration.

As indicated in paragraph 26 of the Craine Declaration, a lower GI tract bleed may result in the production of hematin through the action of bacteria and subsequent exposure to acid. (See page 5, lines 12-16 of the present specification.) The formation of hematin from hemoglobin requires both acid exposure and bacterial action. During a lower GI bleed, the conversion of hemoglobin occurs at highly variable rates that depend upon time in the patient's gut, the bacterial load, etc. The Fielder method exposes these products to an acidic environment (in vitro) that could convert the breakdown products to hematin, erroneously simulating an upper GI bleed. In the present invention, only the result of the acid exposure of hemoglobin in the stomach (in vivo) is measured, and that measured result does not depend upon the highly variable conversion of hemoglobin to hematin. Consequently, the method disclosed in the Fielder reference could give a false positive indication of upper GI tract bleeding and fail to indicate the lower GI tract bleeding, which is the most serious type of error because it could result in failure to diagnose cancer causing the bleeding in the colon. In contrast, action of bacteria does not affect the results of the method of the present invention, because the method of the present invention does not specifically measure hematin.

The Fielder technique for providing a filtered or centrifuged stool sample is completely different from the technique of the described embodiments of the present invention to provide a filtered and centrifuged sample, because in the present invention, filtering is performed to absorb and concentrate the heme components in the nitrocellulose filter and exclude contaminants such as urobilinogen which prevent analysis, so that direct spectroscopic measurement is meaningful,

whereas Fielder centrifuges or filters to remove precipitated hematin. Also, in the present invention, centrifuging is provided to remove stool particles,

Furthermore, ferric hemes other than hematin which are not precipitated also are formed in the upper GI tract. Consequently, there can be an upper GI tract bleed which results in ferric hemes in the stool that are not precipitated and therefore are capable of being detected by the Fielder method and erroneously indicating a lower GI bleed. See paragraph 37 of the Craine declaration.

The Hacker Reference:

The Hacker reference discloses that the hemoglobin degradation products hematin, copratin, and copratoporphyrin appear in feces. Hacker also discloses that the presence of copratoporphyrin is not indicative of an ulcer (i.e., of an upper GI bleed), that copratoporphyrin was found in three of five cases of carcinoma, and that testing for hematin and copratin would be simpler than testing for hematin and copratoporphyrin. The Hacker Reference also discloses a technique in which a large fecal sample is subjected to an extraction procedure using organic solvents, filtered, washed, dried with ethers, and then subjected to another extraction procedure using glacial acetic acid. Specifically, the feces sample is prepared by performing an extraction process with alcohol and ether, filtering, washing with ether, and drying to obtain a powder on which the second extraction procedure is performed with glacial acetic acid to provide a sample for spectroscopic examination.

Thus, the stool sample preparation method disclosed by Hacker is substantially more complex than the stool sample preparation method disclosed by Fielder.

Hacker clearly does not differentiate between upper and lower GI bleeds, but simply identifies the hemoglobin degradation products hematin, copratin and copratoporphyrin which occur in the stool of a person ingesting his/her own blood. Hacker also states that his identifications are not specific for either ulcer or carcinoma. Consequently, Hacker's technique is not specific with regard to the location of GI bleeding.

The approach taught by Hacker uses extractions to purify hematin, copratin, and copratoporphyrin, whereas the described method of the present invention does not use such extractions to purify specific components. Furthermore, an extraction procedure which includes exposure to acid as taught by Hacker would destroy the ferrous forms of heme (through exposure to glacial acetic acid) and would not allow for their measurement which is a key component of the described method of the present invention. See paragraph 39 of the Craine Declaration.

Therefore, it is respectfully submitted that nothing in the Hacker reference suggests any advantage that could be achieved by modifying Fielder's technique to include Hacker's technique of subjecting a large fecal sample to the above mentioned extraction procedure using organic solvents, filtered, washed, dried with ethers, and then subjecting it to the additional extraction procedure using glacial acetic acid to prepare for spectroscopic analysis.

Distinguishing Claims 1 and 5 over Fielder in view of Hacker:

The examiner contends that it would have been obvious for anyone of ordinary skill in the art to apply the spectroscopic approach of Hacker to the method of differentiating between locations of GI bleeding disclosed in Fielder because both references consider the presence of unchanged hemoglobin in a stool sample as an indication of lower GI bleeding and the presence of hematin as a sign of upper GI bleeding, and because the spectrometric approach is simpler and more straightforward. (The applicant agrees with the examiner that using a reference sample without a stool sample to calibrate the spectrophotometer would be obvious.)

Claim 1 recites "collecting a stool sample and preparing it for analysis by spectroscopy". However, although the Fielder reference discloses collecting a stool sample, Fielder clearly does not perform a step of "preparing it for analysis by spectroscopy". Instead, Fielder discloses lowering the pH of the stool sample to 3.5 to 5.0 with a buffer to cause precipitation of hematin and removing the precipitated hematin to prepare the stool sample to be tested for peroxidase activity.

As previously explained, in the described embodiments of the invention, the solid stool sample is put into buffer solution to achieve a very low salt level and to eliminate fecal particulate matter and bacteria from the sample. Since the resulting ionic strength is low, the blood cells in the test sample burst and release many of the hemoglobin breakdown products into the solution so that they can effectively bind to the nitrocellulose filter. See paragraph 27 of the Craine Declaration. It is respectfully submitted that this is much different than, and would not have been suggested by, Fielder's acidifying the stool sample to precipitate and remove hematin.

Therefore, it is respectfully submitted that the Fielder reference would not have disclosed or suggested any advantage to preparing the stool sample for analysis by spectroscopy.

As to the Hacker reference, it simply identifies the hemoglobin degradation products hematin, copratin and copratoporphyrin which occur in the stool of a person ingesting his/her own blood and indicates that the presence of these three degradation products in the stool are not specific for either ulcer or carcinoma. The Hacker reference also discloses the above mentioned sample purification procedure, which is much more complex than the simple, basic sample preparation process disclosed in the Fielder reference. Furthermore, the Hacker reference discloses a method that precludes the direct measurement of ferrous forms of hemoglobin and therefore clearly does not disclose any way of distinguishing between upper and lower GI bleeds.

Therefore, it is respectfully submitted that the Hacker reference does not disclose and would not have suggested any advantage to modifying the Fielder sample preparation procedure by substituting the more complex Hacker sample preparation procedure.

Claim 1 also recites "**determining a sample absorption spectra of the stool sample**". However, Fielder clearly does not determine a sample absorption spectra. Instead, Fielder et al. simply test for peroxidase activity.

It is respectfully submitted that nothing fairly disclosed by the Fielder reference would have provided any suggestion of an advantage of preparing the stool sample for spectroscopy and determining absorption spectra of the stool sample, because the peroxidase diagnostic assay used

in the Fielder reference is simply an enzymatic assay looking for peroxidase activity which converts hydrogen peroxide to oxygen and water. (The oxygen then oxidizes a colorless die, such as gum guaiac, which then turns a purple color revealing the president of the peroxidase activity.)

Hemoglobin is one of a number of substances that have peroxidase activity. However, peroxidase activity is not specific to hemoglobin. See paragraph 28 of the Craine Declaration. The Fielder reference depends upon the peroxidase activity of hemoglobin, but the presence of a number of hemoglobin degradation products cannot be determined by using the peroxidase activity test disclosed in the Fielder reference.

Consequently, a significant problem with the Fielder method is that various other substances have peroxidase activity, including dietary animal myoglobin or vegetable peroxidases (e.g., horseradish peroxidase) so testing for peroxidase activity can result in false positives which erroneously indicate a lower GI bleed and therefore lead to costly and unnecessary follow-up evaluations. See paragraph 29 of the Craine Declaration. It is respectfully submitted that this problem clearly is not recognized by either of the Fielder or Hacker references.

In contrast, the step in **claim 1** of "determining a sample absorption spectra of the stool sample" automatically results in determining the absorption spectra of hemoglobin and a number of hemoglobin breakdown products because the spectroscopic analysis in effect measures the oxidation state of the porphyrin rings regardless of the amount of breakdown of the original

hemoglobin molecule and therefore measures the heme (iron protoporphyrin ring) porphyrin rings not only of the hemoglobin detected by the peroxidase activity test of Fielder, but also the hematin discarded by Fielder and also various other hemoglobin degradation products. See paragraph 30 of the Craine Declaration.

Therefore, it respectively submitted that this advantage of determining the absorption spectra of hemoglobin and a number of its degradation products as a result of using the claimed invention would not have been obvious to an ordinary skill in the art over what is fairly disclosed in the Fielder reference in view of the disclosure of the Hacker reference.

Claim 5 recites "filtering an amount of the fecal extract through a sample filter causing hemoglobin and related molecules present in the fecal extract to adhere to the sample filter", and also recites "determining a sample absorption spectra of the sample filter...". Therefore, the above comments for claim 1 regarding "determining a sample absorption spectra" are equally applicable to **claim 5**.

As to the recitation in **claim 5** regarding "filtering an amount of the fecal extract through a sample filter causing hemoglobin and related molecules present in the fecal extract to adhere to the sample filter", it is respectfully submitted that this limitation clearly is not disclosed in the Fielder reference, because the only filtering disclosed in Fielder is for the purpose of removing precipitated hematin which is not subjected to any testing.

Claim 1 and **Claim 5** recite "determining whether the blood in the stool came from the

upper gastrointestinal site or the lower gastrointestinal site based on an analysis of the sample absorption spectra".

However, Fielder does not disclose "determining whether the blood in the stool came from the upper gastrointestinal site", because Fielder does not test the precipitated hematin. Fielder simply precipitates the hematin and removes it from the solution before using the peroxidase diagnostic assay to determine whether hemoglobin is present in the stool sample. Consequently, Fielder only makes a determination as to whether or not bleeding occurred in the lower GI tract. Fielder does not actually determine if enough hematin was precipitated to indicate an upper GI tract bleed.

Furthermore, is respectfully submitted that nothing in the Fielder reference suggests any advantage to using spectroscopy instead of the disclosed peroxidase diagnostic assay to determine if hemoglobin is present.

Furthermore, measurement of the spectra as described in the present application to indicate the presence of both/either ferrous heme or ferric heme could not be accomplished with Fielder's stool sample because it has been acidified to a pH of 3.5 to 5.0 during preparation of the stool sample. This converts most of the ferrous heme to ferric heme and precludes an accurate characterization of the sample by spectroscopy. See col. 4, lines 1-10 of Fielder. Also see paragraph 31 of the Craine Declaration.

Therefore, it is respectively submitted that the Fielder reference clearly fails to

recognize (1) that the presence of other ferric hemes than hematin is significant to identification of a GI bleed site, and (2) that specialized sample preparation and spectroscopy can allow detection of ferric hemes other than hematin and also of hemoglobin and a number of degradation products thereof.

As to the Hacker reference, it clearly fails to address any of the foregoing matters. Therefore, it is respectfully submitted that Hacker does not suggest any advantage to applying any of its disclosure to that of the Fielder reference.

The Fielder reference distinguishes hematin from hemoglobin by virtue of how hematin precipitates. It is respectfully submitted that this is much different from the present invention, because the present specification describes using spectroscopy of the test sample containing all of the hemoglobin and hemoglobin degradation components to evaluate the chemical condition of the heme portion of molecules that can be in a range of compounds ranging from native hemoglobin to heme which has been highly degraded (by protease activity wherein protein has been "chewed away") but the porphyrin ring is still intact.

The examiner contends that the disclosure of the Hacker reference would have made it obvious to one of ordinary skill in the art to modify the method disclosed in the Fielder reference to use spectroscopy to detect the relative amounts of ferric heme.

However, Fielder precipitates out the hematin sample, which makes it impractical or impossible to use spectroscopy to detect all of the significant ferric hemoglobin degradation

products in the Fielder stool sample.

Furthermore, the Fielder liquid stool samples make it impractical to use spectroscopy to detect ferric content because there are too many interfering substances, such as urobilinogen, that would obscure the results of spectral analysis.

In view of all the above considerations, it is respectfully submitted that claims 1 and 5 would not have been obvious to one of ordinary skill in the art over the Fielder reference in view of the Hacker reference.

Distinguishing dependent claims 2-4 and 6-8 over Fielder in view of Hacker and Brady:

Claims 2-4 and claims 6-8 are dependent on claims 1 and 5, respectively, which are believed to be patentable over the Fielder and Hacker references for the reasons set forth above.

The Brady Reference:

Regarding dependent claims 2-4 and 6-8, the examiner contends that it would have been obvious for anyone of ordinary skill in the art to detect the presence or absence of peaks at 540 nm, 576 nm and 415 nm and for a Soret at 407-408 nm as disclosed by Brady in the Fielder-Hacker method because the first set of absorptions is characteristic for hemoglobin, while the Soret line at 408-409 nm is characteristic for hematin.

However, it is respectfully submitted that no one skilled in the art would have applied the teachings of Brady to the Fielder-Hacker method because the treatments used in the Fielder-Hacker method would chemically destroy the ferrous forms of heme and eliminate the absorption properties associated with those forms. See paragraph 23 of the Craine Declaration.

Also, it is respectfully submitted that no one skilled in the art would ordinarily have looked to any published document to obtain reference values for absorption peaks of ferric heme and ferrous heme with which to compare the spectra of a test stool sample. This is because anyone skilled in the art wanting to use spectroscopy to measure the presence of ferric heme and/or ferrous heme in a stool sample would have simply prepared reference samples of ferric heme and/or ferrous heme at the pH of the test stool sample and measure the reference samples to obtain the corresponding reference absorption peaks. See paragraphs 6 and 32 of the Craine Declaration.

Furthermore, the Brady Reference clearly does not disclose or suggest any of the steps of independent parent claims 1 and 5, which for the reasons already set forth above would have been unobvious to one of ordinary skill in the art at the time of the invention.

In any case, Brady merely discloses spectral peak information for the enzyme L-tryptophan-2,3-dioxygenase. It is respectfully submitted that it would not have been obvious to one of ordinary skill in the art that absorption peaks found by Brady for the enzyme L-tryptophan-2,3-dioxygenase would be the same as those for hemoglobin or hemoglobin derived products because they are completely different molecules. See paragraph 38 of the

Craine Declaration.

Regarding independent claim 9 and dependent claims 14-15 and 20-21, the examiner rejected them as obvious over the Fielder reference in view of the Hacker and Machida references.

Distinguishing independent claim 9 and dependent claims 14-15 and 20-21 over Fielder in view of Hacker and Machida:

The previous comments to distinguish claim 1 over the Fielder reference in view of the Hacker references are equally applicable to claim 9. The Machida reference is discussed below.

The Machida Reference:

The examiner contends that it would have been obvious to use the nitrocellulose filter because "Machida indicates its conventional usage for analysis of biological samples", including either "fecal extracts or deposits on the filter for spectrometric analysis because both ways are conventional for this type of analysis and purify the samples using conventional biochemical separation methods".

However, the Machida reference discloses using the nitrocellulose filter to bind antibody molecules. The solution then is allowed to move across the nitrocellulose filter by capillary action or absorption, and if there is hemoglobin in the solution, it selectively binds to the

antibody that is already bound to the nitrocellulose. See paragraph 33 of the Craine Declaration.

In contrast, in the present invention the hydrophobic properties of the nitrocellulose filter are used to directly bind the heme containing hemoglobin and/or hemoglobin breakdown products. The nitrocellulose filter material selectively adheres to, and therefore concentrates, the ferric heme material and also the ferrous heme material onto the nitrocellulose material. It is respectfully submitted that this is entirely different than indirectly binding only hemoglobin (but not hemoglobin breakdown products) to antibody molecules as disclosed in the Machida reference.

Furthermore, in the described embodiments of the present invention, urobilinigen, the major contaminant in stool, does not bind to nitrocellulose filters and is washed away, thereby providing substantial purification and thereby allowing effective analysis by conventional spectrophotometry.

The method disclosed in the Machida reference would not work in the present invention because the antibody disclosed therein is specific for hemoglobin and would not bind the ferric heme breakdown products. Furthermore, the antibody would bind too little of the hemoglobin to be easily detected by spectrophotometry. See paragraph 34 of the Craine Declaration.

Therefore, it is respectfully submitted that the Machida reference would not have suggested any advantage or reason to use its nitrocellulose filter to prepare the Fielder samples for spectroscopy.

Regarding dependent claims 10-12 and 22, the examiner rejected them as obvious over Fielder in view of Hacker, Machida, and Brady.

Distinguishing dependent claims 10-12 and 22 over Fielder in view of Hacker, Machida and Brady:

The above comments regarding dependent claims 2-4 (which are dependent on claim 1 and 6-8 (which are dependent on claim 5) are equally applicable to claims 10-12 and 22, which are dependent on claim 9, and should be allowable for the same reasons set forth earlier.

Regarding dependent claim 13, the examiner rejected it as obvious over Fielder in view of Hacker, Machida and Schmitz.

Claim 13 is dependent on claim 9, which is believed to be patentable over Fielder in view of Hacker and Machida for the reasons set forth above. As indicated in paragraph 35 of the Craine Declaration, the Schmitz reference does not suggest any advantage of using TE buffer in preparing a stool sample, and more particularly does not suggest an advantage to mixing the stool sample with TE buffer to provide a lower ionic strength than typical "biological conditions" to thereby cause red blood cells to swell and burst and thereby release the hemoglobin and its degradation products into solution.

Therefore, it is respectfully submitted that the use of TE buffer in combination with the limitations of claim 9 would not have been obvious to one of ordinary skill in the art in view of

anything fairly disclosed in the Schmitz reference.

Regarding dependent claim 16, the examiner rejected it as obvious over the Fielder reference in view of the Hacker, Machida, and HYDROFLUOR-Combo reference.

Claim 16 is dependent on claim 9, which is believed to be patentable over the Fielder reference in view of the Hacker and Machida references for the reasons set forth above. The **Hydrofluor-Combo reference** is discussed below:

The Hydrofluor-Combo reference discloses a dehydration treatment involving exposure of cellulose acetate filters to increasing concentration of alcohol in 5% glycerol, followed by using 2% 1,4 diazabicyclo [2.2.2] octane mixed in greater than 95% glycerol combined with a 20 minute heating treatment to clarify the filter. However, the Hydrofluor-Combo reference does not disclose simply using glycerol in a buffer to clarify a filter, and only discloses a more involved procedure also including the octane and heating. Note that the present inventor found that highly diluted glycerol was not effective. See paragraph 36 of the Craine Declaration.

The procedure disclosed in the Hydrofluor-Combo reference would not be suitable for the present invention due to the need for a heat treatment, the high viscosity of the final reagent, and the amount of time required. Only after trial and error, including experiments with other solvents and experiments in which nitrocellulose filters were wetted with a range of glycerol concentrations and the filter translucency measured was a suitable concentration of glycerol found that was effective for making the sample filter more transparent in accordance with the

present invention. See paragraph 36 of the Craine Declaration.

Therefore, it is respectfully submitted that the Hydrofluor-Combo reference would not have disclosed or suggested any advantage is to using 60 percent glycerol by volume sample buffer to increase the transparency of the nitrocellulose sample filter as recited in claim 16.

Regarding dependent claims 16-19, the examiner rejected them as obvious over Fielder in view of the Hacker, Machida, and "Manual for Biochem 651" references and well-known mathematical algorithms for treating complex spectra.

Distinguishing dependent claims 16-19 over Fielder in view of the Hacker, Machida, and "Manual for Biochem 651" references and well-known mathematical algorithms for treating complex spectra:

Claims16-19 are dependent on claim 9, which is believed to be patentable over the Fielder reference in view of the Hacker and Machida references for the reasons set forth above.

Furthermore, the present application describes determining the spectra of the iron protoporphyrin ring to measure whether the heme in the test stool sample is ferrous heme or ferric heme. The spectra indicate relative amounts of ferric vs. ferrous porphyrin rings which are part of the hemoglobin molecule or of a number of hemoglobin degradation products. The spectra is a summation of the spectra for ferric heme and ferrous heme, and the relative amounts can be determined by mathematical methods as set forth on pages 20 and 21 of the specification

to determine the fraction of the individual spectra needed to cause the observed spectrum, wherein in the equation shown on page 20, x_1 indicates the amount of ferrous heme and x_2 indicates the amount of ferric heme. Those quantities can be obtained by solving the system of equations shown on page 21, and the relative amounts of ferrous and ferric heme then can be obtained as a ratio between x_1 and x_2. Spectra are typically plotted as absorption versus wavelength, as in Fig. 2 of the specification, and those skilled in the art typically are able to visually determine the presence of ferric or ferrous forms by pattern recognition. For example the presence of the peaks at 540 nm and 576 nm are easily recognized features of ferrous forms of the molecule. The presence of a broad Soret band or a Soret band with a dip in the middle is recognized as a mixture of ferrous and ferric forms. The heights of the 540 nm peak or the 576 nm peak relative to the height of the Soret band may also serve as a visual cue for a mixture of ferrous and ferric forms. The position of the Soret band can also be visually determined to indicate a predominance of ferric or ferrous forms. See paragraph 18 of the Craine Declaration.

It is respectfully submitted that none of this is disclosed in any of the cited references considered individually, and therefore it is respectfully submitted that none of the references considered individually suggest any advantage to combining their individual teachings as suggested by the examiner to either use spectroscopy in combination with the other steps recited in parent claim 9 or to apply a mathematical analysis to provide the method of the invention as recited in claims 16-19.

In view of the above arguments, it is respectfully submitted that none of the claims would have been obvious to one of ordinary skill in the art over the cited references. Therefore, it is

respectfully submitted that the application now is in condition for allowance.

Respectfully submitted,

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